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Human Endogenous Retrovirus K10 Encodes a Functional Integrase

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We cloned a human endogenous retrovirus K10 DNA fragment encoding integrase and expressed it as a fusion protein with *Escherichia coli* maltose-binding protein. Integrase activities were measured in vitro by using a double-stranded oligonucleotide as a substrate mimicking viral long terminal repeats (LTR). The fusion protein was highly active for both terminal cleavage and strand transfer in the presence of Mn^{2+} on the K10 LTR substrate. It was also active on both Rous sarcoma virus and human immunodeficiency virus type 1 LTR substrates, whereas Rous sarcoma virus and human immunodeficiency virus type 1 integrases were active only on their corresponding LTR substrates. The results strongly suggest that K10 encodes a functional integrase with relaxed substrate specificity.

Human endogenous retroviruses (HERVs) constitute a group of DNA elements identified in the human genome by their homology to vertebrate retroviruses (3–5, 16, 17, 19, 20, 22–25, 27, 30). There seem to exist at least six different sequence families with various degrees of homology among each other (5, 8, 20). The HERV-K family, whose members contain primer-binding sites for $tRNA^{Lys}$, consists of approximately 30 full-length genomes and a few with large deletions (20, 22, 23). HERV-K expresses Gag proteins and sometimes retrovirus-like particles in human cells (2, 8, 10, 14, 15). The prototype of the HERV-K family is HERV-K10, which was detected by Southern blot analysis with the *pol* region of a Syrian hamster intracisternal type A particle genome (22, 23). Its 9.2-kb proviral DNA contains open reading frames (ORFs) capable of encoding proteins related to Gag, Pol, and Env (14, 21, 23), whereas the other known HERV sequences have ORFs that are shorter because of multiple stop codons (30). Although the original HERV-K10 has split ORFs for Gag protein, an variant with a full-length *gag* gene due to a point mutation has been reported. The *gag* and *prt* region of the variant, when expressed in *Escherichia coli*, yields a full length 73-kDa Gag-like polyprotein, which is cleaved by autoproteolysis (21). Processing of the HERV-K10 Gag protein is also known to occur in a human teratocarcinoma-derived cell line (2, 14). Despite the information on Gag expression, little is known about HERV-K Pol (reverse transcriptase and integrase) expression, which is essential for the HERVs to be infectious and capable of retrotransposition.

To examine the biological activity of HERV-K, we have cloned an HERV-K10 DNA fragment encoding putative integrase and expressed it as a fusion protein in *E. coli*. This report describes the expression and characterization of the HERV-K10 integrase (HERVIN) fusion protein.

DNA for a putative HERVIN gene was cloned by PCR with a cloned HERV-K10 subfragment containing ORF4 as a template. ORF4 was chosen as a candidate for integrase because ORF4 encodes a protein homologous to the mouse mammary tumor virus Pol protein (23) and containing two motifs common to retroviral integrases, HHCC and D,D(35)E (11, 13)

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588  ALIKAQELHALTHVNAAGLKNKFDVTWKQAKDIVQHCTQCQVLHLPTQEAGVNPRGLCPN
     ALWQMDVTHVPSFGRLSYVHVTVDTYSHFIWATCQTGESTSHVKKHLLSCFAVMGVPEKI 707
708  KTDNGPGYCSKAFQKFLSQWKISHTTGIPYNSQGQAIVERTNRTLKTQLVKQKEGGDSKE
     CTTPQMQLNLALYTLNFLNIYRNQTTTSAEQHLTGKKNSPHEGKLIWWKDNKNKTWEIGK 827
828  VITWGRGFACVSPGENQLPVWLPTRHLKFYNEPIGDAKKRASTEMVTPVTWMDNPIEVYV
     NDSIWVPGPIDDRCPAKPEEEGMMINISIGYRYPPICLGRAPGCLMPAVQNWLVEVPTVS 947
948  PISRFTYHMVSGMSLRPRVNYLQDFSYQRSLKFRPKGKPCPKEIPKESKNTEVLVWEECV
     ANSAVIL
          1014
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FIG. 1. Amino acid sequence of HERVIN. HERV-K10 (9,178 bp) has several ORFs. The fourth ORF (ORF4), which extends from nt 3878 to 6919 of HERV-K10, is homologous to the mouse mammary tumor virus *pol* gene (23). Part of the amino acid sequence of ORF4 deduced from the reported HERV-K10 sequence (23) is shown. L-HERVIN consists of 427 amino acid residues from 588 to 1014 of ORF4. Underlined letters represent S-HERVIN, which consists of 288 amino acid residues from 588 to 875 of the ORF4. Boldface letters represent conserved amino acid residues among retroviral integrases.

(Fig. 1). By analogy to Rous sarcoma virus (RSV) integrase (RSVIN), amino acid residues 588 and 875 of ORF4 were selected as the first and the last amino acids of a small HERVIN (S-HERVIN; 288 amino acid residues). The last amino acid residue of ORF4 was also selected as the last amino acid residue of a large HERVIN (L-HERVIN; 427 amino acid residues). Two DNA fragments were amplified by PCR with two pairs of oligonucleotides as primers: oligonucleotides 1 (5′-GAG GAT CCG CAC TCA TAA AAG CAC AA-3′;

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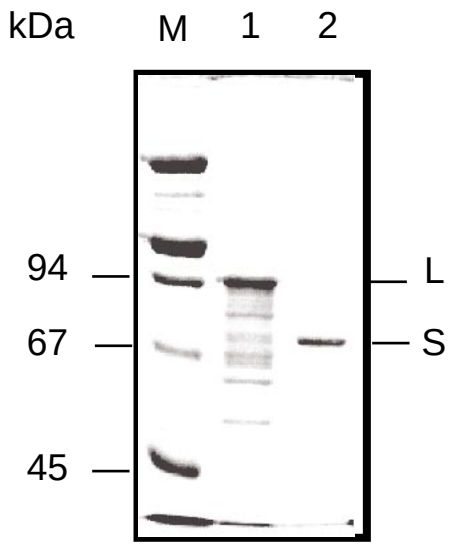


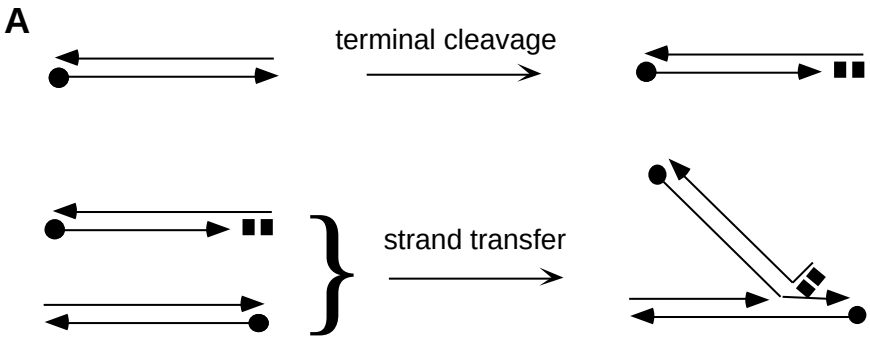
FIG. 2. SDS-polyacrylamide gel electrophoresis analysis of purified MBP fusion proteins of HERVINs. One microgram each of MBP-L-HERVIN (lane 1) and MBP-S-HERVIN (lane 2) was loaded on an SDS–7.5% polyacrylamide gel and stained with Coomassie blue after electrophoresis. Lane M, molecular size markers. Molecular sizes are indicated at the left.

nucleotides [nt] 5639 to 5656 of HERV-K10 are underlined) and 2 (5′-GAG TCG ACT TAT GGT GTT ACC ATC TCC GTG-3′; nt 6502 to 6481 are underlined) for 864 bp of DNA (from nt 5639 to 6502) encoding S-HERVIN and oligonucleotides 1 and 3 (5′-GAG TCG ACT TAT AAT ATC ACC GCA CTA TTG-3′; nt 6919 to 6901 are underlined) for 1,281 bp of DNA (from nt 5639 to 6919) encoding L-HERVIN. The S-HERVIN amino acid sequence was about 40 and 50% identical to the sequences of the reported RSVIN and a potential mouse mammary tumor virus integrase, respectively, whereas it was about 30% identical to the human immunodeficiency virus (HIV) integrase (HIVIN) sequence (5, 7, 12, 23).

The L- and S-HERVINs were expressed as *E. coli* maltose-binding protein (MBP) fusion proteins and purified. After digestion with *Bam*HI and *Sal*I, PCR-amplified DNA fragments for the S- and L-HERVIN genes were inserted into pMAL-c2 (New England Biolabs Inc., Beverly, Mass.) to express HERVIN as a fusion protein with MBP. MBP-HERVINs were expressed in DH5α (Gibco BRL, Gaithersburg, Md.) and purified by affinity column chromatography for MBP as recommended by the manufacturer (New England Biolabs). The final preparations were dialyzed against buffer containing 20 mM sodium *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES-Na) (pH 7.5), 200 mM NaCl, 10% (vol/vol) glycerol, and 1 mM dithiothreitol. The protein concentration was determined by Coomassie blue staining of sodium dodecyl sulfate (SDS)-polyacrylamide gels after electrophoresis with bovine serum albumin as a standard. Purified MBP-L-HERVIN displayed the major 100-kDa polypeptide expected as well as a series of smaller polypeptides (Fig. 2, lane 1), whereas the purified MBP-S-HERVIN preparation yielded a single band of the expected size (70 kDa) (Fig. 2, lane 2). The cause of the partial proteolysis and/or premature translation termination of MBP-L-HERVIN was not further investigated.

As shown in Fig. 3, the purified S- and L-HERVINs were active on an HERV long terminal repeat (LTR) substrate in a terminal cleavage reaction (7), the scheme of which is shown in Fig. 3A. To prepare the blunt-ended HERV LTR substrate whose 22-nt sequence is exactly the same as that of the U3 terminus of the HERV K-10 LTR, oligonucleotide HERV(22−) was 5′ labeled with [γ-^{32}P]ATP (E. I. du Pont de Nemours & Co. [NEN], Boston, Mass.) by using T4 polynucleotide kinase (NEN) at a specific activity of 10^6 cpm/pmol and was annealed to unlabeled oligonucleotide HERV(22+) (Fig. 3B). The reaction was carried out at 37°C for 30 min in 15 μl of reaction mixture containing 33 nM LTR substrate, 20 mM morpholinepropanesulfonic acid (pH 7.2), 10 mM $MnCl_2$, 10 mM 2-mercaptoethanol, 10% (wt/vol) glycerol, 20 mM NaCl, 0.01% bovine serum albumin, 0.75 mM 3-[(3-cholamidopropyl)-dimethyl-ammonio]-1-propanesulfonate (CHAPS), and various amounts of S- or L-MBP-HERVIN. The integration products were analyzed by 15% denaturing polyacrylamide gel electrophoresis followed by autoradiogram analysis with a BAS2000 image analyzer (Fuji Photo Film, Co., Ltd., Tokyo, Japan). The images presented were generated with Photoshop (Adobe Systems, Inc., Mountain View, Calif.) and CANVAS (Deneba Software, Inc., Miami, Fla.) software run on a Macintosh Centris 650 (Apple Computer, Inc., Cupertino, Calif.). Figure 3C shows the terminal cleavage activities of the S- and L-HERVINs at various enzyme concentrations along with the activity of MBP-LacZα, a negative control protein synthesized by pMAL-c2 (New England Biolabs) in DH5α and purified as described above. MBP-LacZα was not active on the HERV LTR substrate (Fig. 3C; compare lanes 1 and 2), whereas at the lowest concentration the S- and L-HERVINs removed 2 nt from the great majority of LTR substrates, indicating that the fusion proteins were similarly active at these concentrations (Fig. 3C, lanes 5 to 14) and that the observed activity was not due to contaminated nucleases. S-HERVIN cleaved the substrate by 1 nt, generating a 21-nt product in addition to a major 20-nt product (Fig. 3C, lanes 9 to 14). It is unclear at present whether or not 1-nt-cleaving activity is inherent in HERVIN. As both S- and L-HERVINs could cleave the HERV ends with almost the same efficiency, S-HERVIN, which is purer than L-HERVIN (Fig. 2), was used for further analyses as described below.

The S-HERVIN was active in both terminal cleavage (Fig. 4A) and strand transfer (Fig. 4B) when tested in vitro on the HERV, RSV, and HIV type 1 (HIV-1) LTR substrates (Fig. 3B). Like the HERV LTR substrate, the 22-nt sequences of the RSV and HIV-1 LTR substrates are the same as the U3 terminus of the RSV LTR and the U5 terminus of the HIV-1 LTR, respectively. S-HERVIN was assayed for terminal cleavage reaction under the conditions described above, with 0.4 μM S-HERVIN; 10 mM Mg^{2+}, Mn^{2+}, or Zn^{2+}; and blunt-ended substrates prepared by annealing of 5′-end-labeled HERV(22−), RSV(22−), and HIV(22−) to HERV(22+), RSV(22+), and HIV(22+), respectively (Fig. 3B). S-HERVIN cleaved the HERV substrate efficiently by 2 nt from the 3′ end in the presence of Mn^{2+} (Fig. 4A, lane 3), whereas it was much less active in the presence of Mg^{2+} (Fig. 4A, lane 2) or Zn^{2+} (Fig. 4A, lane 4). In the presence of Mn^{2+}, S-HERVIN efficiently cleaved the RSV LTR (Fig. 4A, lane 7), and also, although less efficiently, cleaved the HIV-1 LTR substrate (Fig. 4A, lane 11). For the RSV and HIV-1 substrates, S-HERVIN was much less active in the presence of either Mg^{2+} (Fig. 4A, lanes 6 and 10) or Zn^{2+} (Fig. 4A, lanes 8 and 12) and was completely inactive in the absence of divalent cations (Fig. 4A, lanes 1, 5, and 9). Next, S-HERVIN was subjected to strand transfer reactions with the precleaved substrates prepared by annealing 5′-end-labeled HERV(20−), RSV(20−), and HIV(20−) to the HERV(22+), RSV(22+), and HIV(22+) oligonucleotides, respectively (Fig. 3B), under the conditions described for terminal cleavage reactions (Fig. 4A). As shown in Fig. 4B, S-HERVIN acted efficiently in strand transfer on



B

RSV LTR:
5′-AATGTAGTCTTATGCAATACGC-3′ ; RSV(22+)
3′-TTACATCAGAATACGTTATGAG-5′ ; RSV(22-) or RSV(20-)

HIV LTR:
5′-ACTGCTAGAGATTTTCCACACT-3′ ; HIV(22+)
3′-TGACGATCTCTAAAAGGTGTGA-5′ ; HIV(22-) or HIV(20-)

HERV LTR:
5′-AATGTGGGGAAAAGCAAGAGAG-3′ ; HERV(22+)
3′-TTACACCCCTTTTCGTTCTCTC-5′ ; HERV(22-) or HERV(20-)

Comparison of the three sequences
5′-AATGTGGGGAAAAGCAAGAGAG-3′ : HERV(22+)
5′-AATGTAGTCTTATGCAATACGC-3′ : RSV(22+)
5′-ACTGCTAGAGATTTTCCACACT-3′ : HIV(22+)

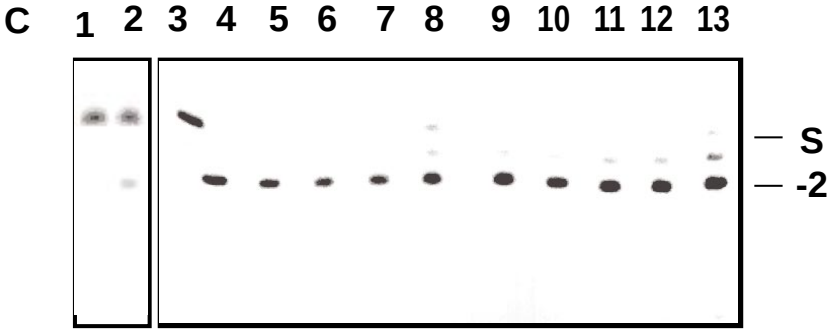


FIG. 3. In vitro integration assay for HERVINs. (A) Schematic diagram of the assay of terminal cleavage and strand transfer activities. Double-stranded oligonucleotides are indicated by arrows. Labeled 5′ ends are indicated by closed circles. Each arrowhead indicates the 3′ end of the strand. In the presence of HERVIN, 2 nt are removed from the 3′ end that mimics one end of the unintegrated linear viral cDNA (7). The cleaved oligonucleotide substrate with protruding 2-nt (closed squares) integrates into another molecule to generate a Y-shaped molecule, producing a labeled oligonucleotide longer than 20 nt. (B) Oligonucleotide substrates used for the in vitro integration assay. Two nucleotides of the 3′ end of each minus strand are cleaved by integrase. For blunt-ended LTR substrates, plus and minus strands of 22 nt each were annealed, while 20-nt minus strands and their corresponding 22-nt plus strands were annealed for precleaved substrates. The viral cDNA ends of HERV, RSV, and HIV-1 (only the sense strand) are aligned for comparison. (C) Terminal cleavage activities of HERVINs. Radiolabeled blunt-ended LTR substrate of HERV-K10 was incubated with various amounts of MBP fusion proteins with LacZα (lane 1), the L-HERVIN (lanes 3 to 8), and the S-HERVIN (lanes 2 and 9 to 13). Each reaction mixture contained 6.7 μM (lanes 1, 3, 4, and 9), 1.7 μM (lanes 5 and 10), 0.42 μM (lanes 6 and 11), 0.11 μM (lanes 2, 7, and 12), or 0.027 μM (lanes 8 and 13) integrase. All the reaction mixtures except that in lane 3 contained 10 mM $MnCl_2$ as a divalent cation. Lane 3 contained 10 mM EDTA instead of $MnCl_2$. S and −2, 22- and 20-nt oligonucleotides, respectively.

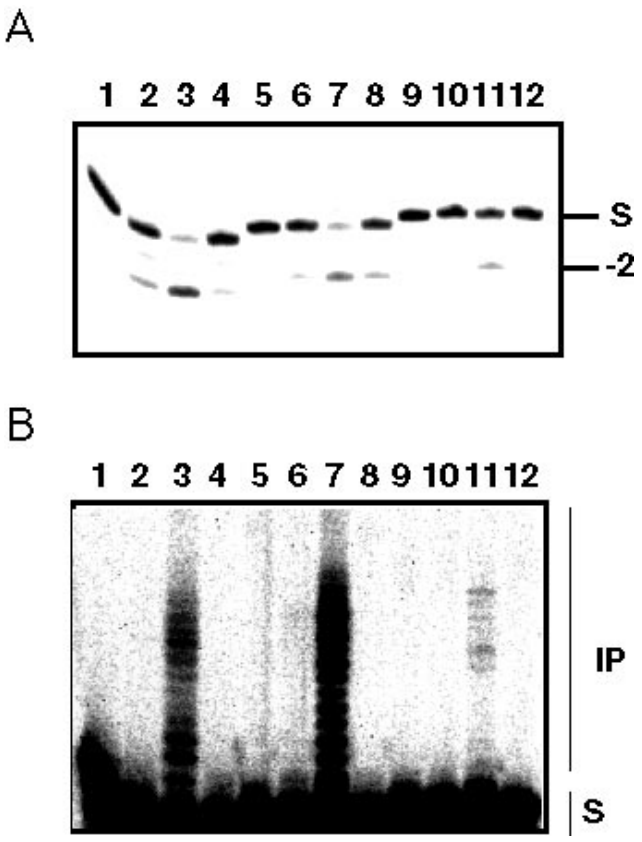


FIG. 4. Terminal cleavage and strand transfer activities of S-HERVIN. (A) Terminal cleavage activity of HERVIN. Analysis of terminal cleavage products by denaturing polyacrylamide gel electrophoresis is shown. The reactions were carried out under the same conditions as described for Fig. 3C, with 0.42 μM purified MBP-S-HERVIN fusion protein with blunt-ended LTR substrate of either HERV (lanes 1 to 4), RSV (lanes 5 to 8), or HIV-1 (lanes 9 to 12). The reaction mixtures contained 10 mM either EDTA (lanes 1, 5, and 9), $MgCl_2$ (lanes 2, 6, and 10), $MnCl_2$ (lanes 3, 7, and 11), or $Zn(OCOCH_3)_2$ (lanes 4, 8, and 12). S and −2, 22- and 20-nt oligonucleotides, respectively. (B) Strand transfer activity of HERVIN. Strand transfer products were analyzed by denaturing polyacrylamide gel electrophoresis. Reaction conditions and the order of the lanes are the same as for panel A except that precleaved LTR substrates were used. IP and S, integration products and substrate, respectively.

the HERV and RSV substrates and, though less efficiently, on the HIV-1 substrate in the presence of Mn^{2+} (Fig. 4B, lanes 3, 7, and 11), generating a series of oligonucleotides longer than 22 nt (Fig. 3A for a schematic). S-HERVIN showed much less efficient strand transfer activity in the presence of Mg^{2+} (Fig. 4B, lanes 2, 6, and 10) or Zn^{2+} (Fig. 4B, lanes 4, 8, and 12) and was inactive in the absence of divalent cations (Fig. 4B, lanes 1, 5, and 9).

Unlike HERVIN, RSVIN and HIVIN were active in both terminal cleavage and strand transfer only on their own corresponding substrates. The MBP fusion proteins with RSVIN and HIVIN, prepared as described previously (11, 12), were subjected to an in vitro integration reaction to test whether they have similar relaxed donor sequence specificities. The reaction conditions were the same as described above for Fig. 3C and 4, with 0.4 μM MBP fusion protein with RSVIN or HIVIN in place of HERVIN. Although our preparation of MBP-RSVIN appeared to be less active than MBP-HIVIN, the two proteins could cleave only their own corresponding blunt-ended substrates (data not shown). In strand transfer, MBP-RSVIN was active on its corresponding precleaved substrate (Fig. 5A, lane 7) in the presence of Mn^{2+}, whereas it showed little strand transfer activity on the HERV or HIV substrate (Fig. 5A, lanes 3 and 11). Similarly, MBP-HIVIN showed efficient strand transfer activity on its corresponding substrate (Fig. 5B, lane 11) in the presence of Mn^{2+}, whereas it was inactive in strand transfer on the RSV or HERV LTR substrate (Fig. 5B, lanes 1 to 8). The two proteins were much less active in the presence of Mg^{2+} and Zn^{2+} (Fig. 5A, lanes 2, 4,

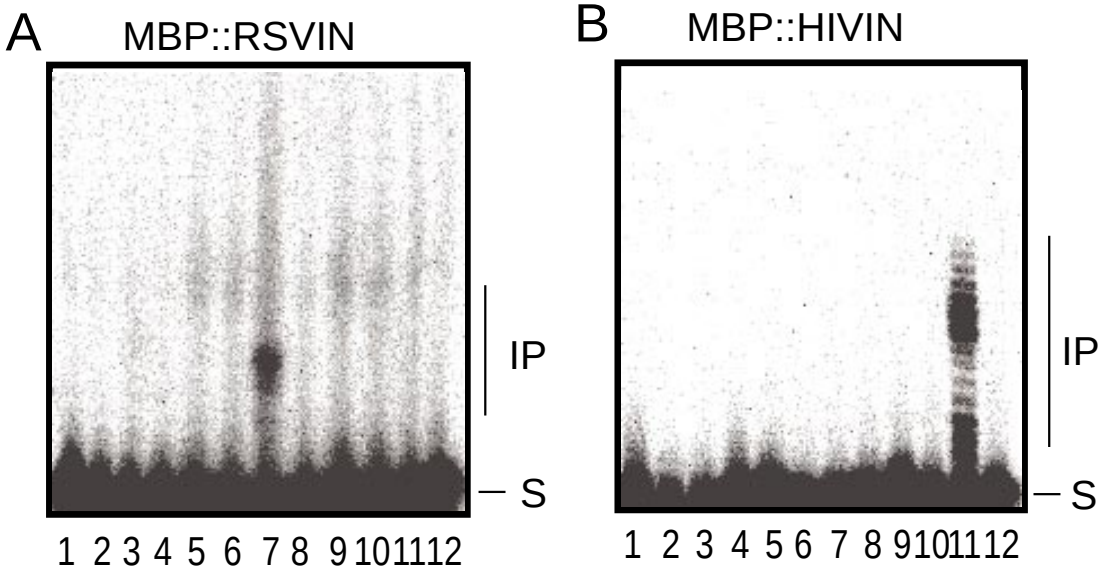


FIG. 5. Strand transfer activities of RSVIN and HIVIN. The strand transfer activities of MBP-RSVIN (A) and MBP-HIVIN (B) were analyzed in vitro by using HERV, RSV, and HIV substrates. Reaction conditions are the same as described for Fig. 4B, with the MBP fusion protein with RSVIN or HIVIN in place of HERVIN. For both panels, precleaved LTR substrates of either HERV (lanes 1 to 4), RSV (lanes 5 to 8), or HIV-1 (lanes 9 to 12) were used. The reaction mixtures contained 10 mM either EDTA (lanes 1, 5, and 9), $MgCl_2$ (lanes 2, 6, and 10), $MnCl_2$ (lanes 3, 7, and 11), or $Zn(OCOCH_3)_2$ (lanes 4, 8, and 12). IP and S, integration products and substrate, respectively.

6, 8, 10, and 12, and Fig. 5B, lanes 2, 4, 6, 8, 10, and 12). Thus, HIVIN and RSVIN appeared to be capable of specifically recognizing their corresponding substrates.

In this study, HERVIN was expressed as MBP fusion proteins in *E. coli* and was characterized in vitro. Purified HERVIN was shown to have both terminal cleavage and strand transfer activities on HERV, HIV, and RSV LTR substrates (Fig. 4) but was not active on an unrelated oligonucleotide substrate which is 22 nt long with the same base composition as the HERV substrate (data not presented). The relaxed donor sequence requirement is a remarkable characteristic of HERVIN, because HIVIN and RSVIN were active only on their corresponding LTR substrates (Fig. 5) and because the three LTR sequences are considerably different (Fig. 3B). Apparently, HERVIN resembles feline immunodeficiency virus (FIV) integrase (FIVIN) in that FIVIN cleaves and integrates both the FIV and HIV-1 LTR substrates (29a). However, HERVIN is different from FIVIN in that HIVIN is active on viral LTR substrate of FIV but not on that of HERV. That is, FIVIN and HIVIN are exchangeable, whereas HERVIN is not exchangeable with HIVIN or RSVIN. The common property of HERVIN, HIVIN, and RSVIN is divalent-cation dependence; purified HERVIN, HIVIN, and RSVIN were active in the presence of Mn^{2+}, whereas they were less active in the presence of Mg^{2+} or Zn^{2+} (Fig. 4 and 5).

Possibly, HERV is one of the agents involved in pathogenesis of seminomas (26) and immunological disorders (1, 9, 28, 29), and its pathogenic activity may require retrotransposition or virus production. Active integrase, together with reverse transcriptase, is essential for HERV to be capable of retrotransposition and to give rise to infectious viruses. An integrase function may be available, as shown in this study, in human cells, whereas a reverse transcriptase function may be supplied in *trans* by a human long interspersed nuclear element (6, 18) even if HERV lacks reverse transcriptase activity. However, whether HERV can retrotranspose and trigger insertional mutagenesis and whether HERV can be activated to replicate in human cells remain to be investigated.

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REFERENCES

1. **Abraham, G. N., and A. S. Khan.** 1990. Human endogenous retroviruses and immune disease. Clin. Immunol. Immunopathol. **56:**1–8.
2. **Boller, K., H. König, M. Sauter, N. Mueller-Lantzsch, R. Löwer, J. Löwer, and R. Kurth.** 1993. Evidence that HERV-K is the endogenous retrovirus sequence that codes for the human teratocarcinoma-derived retrovirus HTDV. Virology **196:**349–353.
3. **Callahan, R., I. M. Chiu, J. F. Wong, S. R. Tronick, B. A. Roe, S. A. Aaronson, and J. Schlom.** 1985. A new class of endogenous human retroviral genomes. Science **228:**1208–1211.
4. **Coffin, J. M.** 1982. Endogenous viruses, p. 1109–1204. *In* R. Weiss, N. Teich, H. E. Varmus, and J. M. Coffin (ed.), RNA tumor viruses. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
5. **Deen, K. C., and R. W. Sweet.** 1986. Murine mammary tumor virus *pol*-related sequences in human DNA: characterization and sequence comparison with the complete murine mammary tumor virus *pol* gene. J. Virol. **57:**422–432.
6. **Dombroski, B. A., S. L. Mathias, E. Nanthakumar, A. F. Scott, and H. H. J. Kazazian.** 1991. Isolation of an active human transposable element. Science **254:**1805–1807.
7. **Engelman, A., K. Mizuuchi, and R. Craigie.** 1991. HIV-1 DNA integration: mechanism of viral DNA cleavage and DNA strand transfer. Cell **67:**1211–1221.
8. **Franklin, G. C., S. Chretien, I. M. Hanson, H. Rochefort, F. E. B. May, and B. R. Westley.** 1988. Expression of human sequences related to those of mouse mammary tumor virus. J. Virol. **62:**1203–1210.
9. **Gourley, M. F., W. J. Kisch, C. F. Mojcik, L. B. King, A. M. Krieg, and A. D. Steinberg.** 1994. Role of endogenous retroviruses in autoimmune diseases. Tohoku J. Exp. Med. **173:**105–114.
10. **Kato, N., S. Pfeifer-Ohlsson, M. Kato, E. Larsson, J. Rydnert, R. Ohlsson, and M. Cohen.** 1987. Tissue-specific expression of human provirus ERV3 mRNA in human placenta: two of the three ERV3 mRNAs contain human cellular sequences. J. Virol. **61:**2182–2191.
11. **Khan, E., J. P. Mack, R. A. Katz, J. Kulkosky, and A. M. Skalka.** 1991. Retroviral integrase domains: DNA binding and the recognition of LTR sequences. Nucleic Acids Res. **19:**851–860.
12. **Kitamura, Y., Y. M. Lee, and J. M. Coffin.** 1992. Nonrandom integration of retroviral DNA in vitro: effect of CpG methylation. Proc. Natl. Acad. Sci. USA **89:**5532–5536.
13. **Kulkosky, J., K. S. Jones, R. A. Katz, J. P. G. Mack, and A. M. Skalka.** 1992. Residues critical for retroviral integrative recombination in a region that is highly conserved among retroviral/retrotransposon integrases and bacterial insertion sequence transposases. Mol. Cell. Biol. **12:**2331–2338.
14. **Löwer, R., K. Boller, B. Hasenmaier, C. Korbmacher, N. Müller Lantzsch, J. Löwer, and R. Kurth.** 1993. Identification of human endogenous retroviruses with complex mRNA expression and particle formation. Proc. Natl. Acad. Sci. USA **90:**4480–4484.
15. **Löwer, R., J. Löwer, C. Tondera-Koch, and R. Kurth.** 1993. A general method for the identification of transcribed retrovirus sequences (R-U5 PCR) reveals the expression of the human endogenous retrovirus loci HERV-H and HERV-K in teratocarcinoma cells. Virology **192:**501–511.
16. **Mager, D. L., and P. S. Henthorn.** 1984. Identification of a retrovirus-like element in human DNA. Proc. Natl. Acad. Sci. USA **78:**4892–4896.
17. **Martin, M. A., T. Bryan, S. Rasheed, and A. S. Khan.** 1981. Identification and cloning of endogenous retroviral sequences present in human DNA. Proc. Natl. Acad. Sci. USA **78:**4892–4896.
18. **Mathias, S. L., A. F. Scott, H. H. J. Kazazian, J. D. Boeke, and A. Gabriel.** 1991. Reverse transcriptase encoded by a human transposable element. Science **254:**1808–1810.
19. **May, F. E. B., and B. R. Westley.** 1986. Structure of a human retroviral sequence related to mouse mammary tumor virus. J. Virol. **60:**743–749.
20. **Medstrand, P., and J. Blomberg.** 1993. Characterization of novel reverse transcriptase encoding human endogenous retroviral sequences similar to type A and type B retroviruses: differential transcription in normal human tissues. J. Virol. **67:**6778–6787.
21. **Mueller-Lantzsch, N., M. Sauter, A. Weiskircher, K. Kramer, B. Best, M. Buck, and F. Grässer.** 1993. Human endogenous retroviral element K10 (HERV-K10) encodes a full-length gag homologous 73-kDa protein and a functional protease. AIDS Res. Hum. Retroviruses **9:**343–350.
22. **Ono, M.** 1986. Molecular cloning and long terminal repeat sequences of human endogenous retrovirus genes related to types A and B retrovirus genes. J. Virol. **58:**937–944.
23. **Ono, M., T. Yasunaga, T. Miyata, and H. Ushikubo.** 1986. Nucleotide sequence of human endogenous retrovirus genome related to the mouse mammary tumor virus genome. J. Virol. **60:**589–598.
24. **Rabson, A. B., P. E. Steele, C. F. Garon, and M. A. Martin.** 1983. mRNA transcripts related to full-length retroviral DNA in human cells. Nature (London) **306:**604–607.
25. **Repaske, R., P. E. Steele, R. R. O'Neill, A. B. Rabson, and M. A. Martin.** 1985. Nucleotide sequence of a full-length human endogenous retroviral segment. J. Virol. **54:**764–772.
26. **Sauter, M., S. Schommer, E. Kremmer, K. Remberger, G. Dölken, I. Lemm, M. Buck, B. Best, D. Neumann-Haefelin, and N. Mueller-Lantzsch.** 1995. Human endogenous retrovirus K10: expression of Gag protein and detection of antibodies in patients with seminomas. J. Virol. **69:**414–421.
27. **Stoye, J. P., and J. M. Coffin.** 1985. Endogenous viruses, p. 357–404. *In* R. Weiss, N. Teich, H. E. Varmus, and J. M. Coffin (ed.), RNA tumor viruses 2. Supplements and appendixes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
28. **Takeuchi, K., K. Katsumata, H. Ikeda, M. Minami, A. Wakisaka, and T. Yoshiki.** 1995. Expression of endogenous retroviruses, ERV3 and lambda 4-1, in synovial tissues from patients with rheumatoid arthritis. Clin. Exp. Immunol. **99:**338–344.
29. **Talal, N., E. Flescher, and H. Dang.** 1992. Are endogenous retroviruses involved in human autoimmune disease? J. Autoimmun. **5**(Suppl. A)**:**61–66.

29a. **Vink, C., K. H. van der Linden, and R. H. A. Plasterk.** 1994. Activities of the feline immunodeficiency virus integrase protein produced in *Escherichia coli*. J. Virol. **68:**1468–1474.

30. **Wilkinson, D. A., D. L. Mager, and A. C. Leong.** 1994. Endogenous human retroviruses, p. 465–535. *In* A. Levy (ed.), The Retroviridae, vol. 3. Plenum, New York.